

## ANTIBACTERIAL EVALUATION OF *AZADIRACHTA INDICA* ETHANOLIC LEAF EXTRACT AGAINST SELECTED ACIDOGENIC ORAL BACTERIA CAUSING DENTAL PLAQUE IN FIXED ORTHODONTIC APPLIANCE PATIENTS – AN *INVITRO* STUDY

LAKSHMI. T<sup>1</sup> & ARAVIND KUMAR. S<sup>2</sup>

<sup>1</sup>Assistant Professor, Department of Pharmacology, Saveetha Dental College & Hospitals, Tamil Nadu, India

<sup>2</sup>Reader, Department of Orthodontics & Dentofacial Orthopedics, Saveetha Dental College & Hospitals, Tamil Nadu, India

### ABSTRACT

The objective of our study is to evaluate the antibacterial activity of ethanolic leaf extract of *Azadirachta Indica* against selected acidogenic oral bacteria that causes dental plaque in fixed orthodontic appliance patients. *Azadirachta Indica* is used as a traditional medicine from antiquity. Various parts of the plant possess medicinal value for curing diseases. the ethanolic extract of *Azadirachta Indica* leaf were used to find out the antibacterial property against *streptococcus mutans*, *streptococcus mitis*, *streptococcus sanguis*, *streptococcus salivarius* and *Lactobacillus acidophilus* by using the Broth culture method. The extract exhibited significant antibacterial activity against *streptococcus mitis*, *streptococcus mutans*, *streptococcus sanguis* & *streptococcus salivarius* with minimum bactericidal concentration of 250 µg/ml, 500 µg/ml, 1mg/ml & 5mg/ml whereas, the extract does not show antibacterial activity against *Lactobacillus acidophilus*.

**KEYWORDS:** *Azadirachta indica*, Anti Bacterial Evaluation, MBC, Oral Bacteria, Orthodontic Fixed Appliance, Dental Plaque

### INTRODUCTION

The presence of orthodontic appliances impedes oral hygiene measures and alters the oral microbial ecosystem to a more pathogenic oral biofilm. Subsequent accumulation of plaque can contribute to development of chronic periodontal inflammation and can progress to gingival enlargement.<sup>1</sup> Gingival enlargement inhibits hygiene measures, slows down orthodontic tooth movement and cause aesthetic and functional problems. Plaque control can be maintained by brushing, flossing and scaling.

Throughout India rural people use Neem twigs and leaves to brush their teeth, and keep their gums free of disease and infection, even though they have limited access to modern dental care. The ancient Ayurvedic practice of using Neem to heal and rejuvenate gum tissue and to prevent cavities and gum disease is verified in modern clinical studies.<sup>2</sup> study shows that Neem leaf extract is used to treat dental plaque and gingivitis.<sup>3</sup>

The medicinal utilities of neem have been described, especially for leaf, fruit and bark<sup>4</sup>. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation and also as a general health promoter.<sup>5</sup> Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident.<sup>6</sup>

Neem oil finds use to control various skin infections.<sup>7-10</sup> Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis.<sup>11</sup> Neem possess anti-inflammatory<sup>12</sup>, antipyretic activity.<sup>13</sup> neem bark possess Immunostimulant activities.<sup>14</sup> neem leaf possess Hypoglycaemic<sup>15</sup> and anti ulcer effect neem oil produces antifertility effect.<sup>16</sup> neem seed and leaf possess antimalarial<sup>17</sup> and antibacterial activity,<sup>18</sup> Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action Aqueous leaf extract offers antiviral activity against Vaccinia virus -<sup>19</sup>

Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7,12-dimethylbenz[a]anthracene (DMBA), as revealed by reduced incidence of neoplasm.<sup>20</sup> The aqueous extract of neem leaf shows good antioxidant activity.<sup>21</sup> The crude ethanolic extract of stem bark and root bark showed hypotensive, spasmolytic and diuretic activities.<sup>22,23</sup>

Hence an attempt was taken by us to evaluate the antibacterial activity of neem against selected acidogenic oral bacteria responsible for causing dental plaques in fixed orthodontic appliances patients.

## MATERIALS AND METHODS

### Plant Material

Ethanolic leaf extract of *Azadirachta indica* were obtained from Green Chem. Herbal Extracts & Formulations. Bangalore.

### Test Microorganisms

Bacterial strains used were *streptococcus mutans* (ATCC 25175), *streptococcus salivarius* (ATCC 25975), *streptococcus mitis* (ATCC 9811), *streptococcus sanguis* (ATCC 10557), *Lactobacillus acidophilus* (ATCC 4356). The organisms were obtained from Department of Microbiology, Saveetha Dental College & Hospitals, Chennai.

## METHODOLOGY

The plant extract 200mg were weighed aseptically into a sterile tube and dissolved in 2ml of sterile Tryptic soy Broth (TSB). From the stock solution various concentrations were prepared, viz., 62µg, 125µg, 250µg, 500µg/100µl, 1mg, 5mg, 10mg/100µl respectively in to wells of micro plates. 100µl of these concentration were taken and the plates were incubated at 37°C for 24hrs.

## SCREENING OF ANTIBACTERIAL ACTIVITY

The tested organisms was grown in (TSB) Tryptic soy broth medium [Hi media, Mumbai] for 24hrs at 37°C and concentration was adjusted to 0.5 Macfarland standard.<sup>24-26</sup> The above concentration of extracts were taken in 100µl quantities in a U bottom micro culture plates. 100µl of the bacterial suspension was added to each well. control well received plain broth without plant extract. the plates were kept in sealed covers and incubated at 37°C overnight and growth/no growth was detected. All the tests were done in duplicate to minimize the test error.

## MINIMUM INHIBITORY CONCENTRATION (MIC)

Minimum inhibitory concentration of herbal extracts against tested microorganism was determined by broth culture method.<sup>27</sup> A series of two- fold dilution of each extract (62 µg/100µl to 10mg/100µl) was made in to which 100µl of the standardized bacterial suspension containing 10<sup>6</sup> organisms was made in Tryptic soy broth as specified by National

Committee for Clinical Laboratory Standards (NCCLS, 1990)<sup>28</sup> The control well received plain broth without herbal extract. The plates were incubated at 37°C for 24 hours and observed for visible growth. As the extracts were colored, MIC could not be read directly by visual methods. Hence subcultures from all the wells were made and growth/no growth is detected then the MBC were obtained.

### MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The MBCs were determined by selecting wells that showed no growth. The least concentration, at which no growth was observed, were noted as the MBC.

### RESULTS & DISCUSSIONS

In the human oral cavity, *streptococci* are the predominant indigenous microorganisms, especially *Streptococcus sanguis*, which is most important in the early stage of plaque formation. A growth inhibitory substance from *S. sanguis* is therefore of interest, since such a bacteriocin is one of the conceivable survival stratagems for the organisms in the oral microbial population. The primary acid tolerant bacteria associated with dental plaque includes *streptococcus mutans*, *Streptococcus oralis*, *streptococcus sobrinus*, *Lactobacillus acidophilus*, *streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus intermedius*, *Streptococcus anginosus* that surround orthodontic appliances are a common problem in many patients undergoing Orthodontic treatment.<sup>29-33</sup>

Such bacteria can lead to tooth enamel breakdown and potential discoloration of the tooth surface, and these aesthetic changes can persist for many years after orthodontic treatment. While the newer bonded orthodontic brackets have many advantages over the old metal bands that were fitted around each tooth, they do impede good oral hygiene, resulting in plaque accumulation and increased tooth enamel breakdown. It also has been reported that presence of fixed orthodontic appliance greatly inhibits oral hygiene and creates new retentive areas for plaque and debris<sup>34</sup>, which in turn predisposes to increased carriage of microbes and subsequent infection. Therefore, prevention of bacterial attachment to orthodontic wires is a critical concern for orthodontists.<sup>35,36</sup>

Prashanth *et al* conducted a study to evaluate the antimicrobial effects of the chewing sticks of Neem and mango against the oral microbes which are involved in the development of dental caries. He suggested that combination of neem and mango chewing sticks may provide the maximum benefit to mankind to prevent dental caries.<sup>37</sup>

Venka A *et al* conducted a study related to the antibacterial effect of Neem mouthwash against salivary levels of *streptococcus mutans* and *Lactobacillus Acidophilus* has been tested over a period of two months, also its effect in reversing incipient carious lesions was assessed. He found that *streptococcus mutans* was inhibited by Neem mouthwashes, with or without alcohol as well as chlorhexidine, *lactobacillus* growth was inhibited by chlorhexidine alone.<sup>38</sup>

In table 1 *Azadirachta indica* ethanolic leaf extract shows No growth (MBC) at a concentration of 250 µg/ml and 500 µg/ml against *streptococcus mutans* and *streptococcus mitis*. *Azadirachta indica* also shows No growth (MBC) at a concentration of 1mg/ml & 5mg/ml against *streptococcus sanguis* & *streptococcus salivarius*. In conclusion, *Azadirachta indica* leaf extract is highly effective against *streptococcus mutans* and *streptococcus mitis* when compared to the other microorganisms tested. However the extract showed no activity against *lactobacillus acidophilus*.

Our finding suggest the presence of No growth is an indication of high effectiveness of the extract whereas presence of Growth indicates the less effectiveness of the extract, which was represented in Table 1.

**Table 1 : Antibacterial Activity of Azadirachta Indica against Acidogenic Oral Bacteria**

<i>Azadirachta indica</i> Ethanolic Leaf Extract	62 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1mg/ml	5mg/ml	10mg/ml	Control	MBC
<i>Streptococcus mutans</i>	++	++	++	--	--	--	--	++	500µg/ml
<i>Streptococcus mitis</i>	++	++	--	--	--	--	--	++	250µg/ml
<i>Streptococcus sanguis</i>	++	++	++	++	--	--	--	++	1mg/ml
<i>Streptococcus salivarius</i>	++	++	++	++	++	–	–	++	5mg/ml
<i>Lactobacillus acidophilus</i>	++	++	++	++	++	++	++	++	NO ACTIVITY

++ =Growth -- = No Growth

## CONCLUSIONS

Oral diseases are multifactorial in nature. Microbial etiology is well accepted and established as a major etiological factor for oral diseases. Oral microbes evade the host immune response and alter the host inflammatory reaction. Physical removal of plaque by scaling and root planing and removal of dental caries are widely used as a treatment modality.

Prevention of oral diseases like dental caries, dental plaque by natural means is well appreciated. Since herbal medicines possess anti microbial, anti inflammatory activity, it can be effectively used as a prophylactic and therapeutic agent against acidogenic oral bacteria causing dental plaque in fixed orthodontic appliances patients. Compared to other therapeutic agents herbs like neem leaves have lesser side effects, easily available and economical. Randomized controlled clinical trials are needed to evaluate the long term success of these products.

## ACKNOWLEDGEMENTS

Our Heartfelt thanks to Mr. Rajendran CEO Of Green Chem Herbal Extracts & Formulations, Bangalore, India for Providing us the ethanolic leaf extract of *Azadirachta indica* as a gift sample to conduct this *In vitro* Study and we wish to thank Dr. Auxilia Hemamalini ,HOD of Microbiology , Saveetha Dental College & Hospitals, Chennai for providing the test organisms for the study.

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